BRAF Exon 15 T1799A Mutation Is Common in Melanocytic Nevi, but Less Prevalent in Cutaneous Malignant Melanoma, in Chinese Han

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Frequent somatic mutations of *BRAF* (*v-raf murine sarcoma viral oncogene homolog B*) exon T1799A, which are implicated in the initial events of promutagenic cellular proliferation, are detected in both malignant melanomas (MM) and melanocytic nevi (MN). Most of the data regarding *BRAF* exon T1799A mutation have been from Caucasian cohorts, and a comprehensive screening of a homogeneous population is lacking. A total of 379 cases of MN and 195 cases of MM were collected from Chinese Han living in three geographical regions in China, i.e., northeast, southwest, and northwest China. *BRAF* exon T1799A mutation was detected by PCR and sequencing from microdissected tumors. In all, 59.8% cases of MN harbored *BRAF* exon T1799A mutation. Samples from regions with high UV exposure had higher detection rates than regions with lower UV exposure (73.5, 67.0, and 38.9%, respectively; $\chi^2 = 31.674$, $P = 1.59E{-}7$). There were no differences in mutation rates between congenital and acquired MN; however, acquired MN with advanced age of onset had a higher mutation rate than those with younger age of onset ($\chi^2 = 13.23$, $P = 0.02$). In all, 15.0% cases of MM harbored the *BRAF* mutation. The mutation rate in MM was not affected by region, histological type, gender, pattern of UV exposure, and age. The study suggests that the mutation is not necessarily associated with malignant transformation.

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INTRODUCTION

Malignant melanoma (MM) and melanocytic nevus (MN) are, respectively, malignant and benign forms of melanocytic tumors. The incidence of cutaneous MM varies among different races, with Caucasians having the highest rate, Asians a moderate rate, and blacks the lowest rate. The clinical and histological types of MM vary among different ethnicities, such that Caucasians are often afflicted with superficial spreading MM and nodular MM (Lang and MacKie, 2005), whereas Asians present with acral lentiginous MM (Sasaki *et al.*, 2004). Cutaneous MN occurs universally, although more frequently in Caucasians than in dark-skinned Africans and Asians. The incidence of MN is also related to age; there are few cases in early childhood, but the incidence peaks in the third decade of life and then declines again with advancing age (Green and Swerdlow, 1989).

BRAF (*v-raf murine sarcoma viral oncogene homolog B*), a member of the RAF family, is a critical serine/threonine kinase in the RAS/mitogen-activated protein kinase pathway (RAS-RAF-MEK-ERK-MAP kinase pathway). Somatic mutations of *BRAF*, especially T1799A (V600E) in exon 15, have been found at varying levels in cases of MM (Davies *et al.*, 2002; Sasaki *et al.*, 2004; Lang and MacKie, 2005; Poynter *et al.*, 2006; Saldanha *et al.*, 2006; Liu *et al.*, 2007). The mutant *BRAF* promotes melanoma growth and development and has thus been regarded as one of the key underlying causes of primary melanomas (Patton *et al.*, 2005; Hoeflich *et al.*, 2009). Subsequent studies have suggested that the rates of *BRAF* mutations are related to the subtypes of MM (Cohen *et al.*, 2004; Liu *et al.*, 2007; Wu *et al.*, 2007). In addition, there

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Abbreviations: BRAF, v-raf murine sarcoma viral oncogene homolog B; *MM, malignant melanoma; MN, melanocytic nevus;* NRAS, neuroblastoma rat sarcoma oncogene

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is accumulating evidence that MN harbor *BRAF* T1799A mutations, with frequencies reaching as high as 80% (Pollock *et al.*, 2003; Poynter *et al.*, 2006). Transformation of a MN to MM is not uncommon, but the relatively high detection rate of *BRAF* mutation in MN hardly accounts for all the transformations to MM, thus leaving doubt as to the role of *BRAF* mutation in melanoma development (Wu *et al.*, 2007). A recent finding that *BRAF* T1799A mutation alone led to long-term melanocytic hyperplasia in mice favored the hypothesis that *BRAF* mutation is one of the early events leading to melanocytic proliferation in MN and MM (Dankort *et al.*, 2009).

From the clinical perspective, sunlight exposure, especially UV light, is generally regarded as one of the important factors associated with some types of MM and MN (Pollock *et al.*, 2003). Several studies showed that intermittent UV exposure correlated with *BRAF* mutations in MM and MN in fair-skinned people of various ethnicities (Sasaki *et al.*, 2004; Liu *et al.*, 2007; Akslen *et al.*, 2008). The importance of UV exposure to the occurrence of MM or MN is possibly influenced by skin type and color. Therefore, we undertook a retrospective study on *BRAF* mutations in the MN and MM of Chinese Han, a genetically homogeneous population with skin types III and IV. The samples were collected from three regions with distinct geographical conditions.

RESULTS

MN and MM in Chinese Han rarely harbored mutations in *BRAF* exon 11 and *NRAS* exons 2 and 3

Our first-phase study was performed on 280 consecutive samples of MM ($N=109$) and MN ($N=171$) that yielded qualified DNA. Sources of the samples and demographic features of this cohort were similar to those of the larger cohort, as shown in Tables 1 and 5. No mutations in *BRAF* exon 11 were detected in 276 samples (excluding 4 samples where PCR failed). No mutations in *NRAS* (*neuroblastoma rat sarcoma oncogene*) exon 2 were detected in 267 samples (PCR failed in 13 samples). Four mutations in *NRAS* exon 3 were detected in 274 samples (PCR failed in 6 samples). The mutation at *BRAF* exon 15 T1799A was detected in 63.7% (107 of 168) of MN cases and 14.7% (16 of 109) of MM cases. In addition, one case of *BRAF* exon 15 A1781G and one case of G1800A were detected (Table 2). The representative figures are shown in Figure 1. Based on this first-phase study, we discontinued investigating the *BRAF* exon 11 and *NRAS* exon 2 and 3 mutations and concentrated on detection of the *BRAF* exon 15 T1799A mutation in expanded sample groups of patients with MN and MM, as described below.

Chinese Han individuals with MN harbor a high frequency of *BRAF* exon 15 T1799A mutation, although geographical variation exists

Owing to failures in either DNA extraction or PCR, we had 341 cases (out of 379) of MN that qualified for analysis, as shown in Table 1. We detected the *BRAF* T1799A mutation in 204 cases (59.8%) of MN in the overall cohort. We also detected a statistically significant difference in the mutation rate among the three geographical regions ($\chi^2=31.67$, $P=1.59$E–7). By multiple logistic regression analysis, the mutation rate was significantly lower in MN from northeast China than those from northwest China (38.9 vs. 67.0%, odds ratio (OR) = 3.88, 95% confidence interval (CI) = 2.17–6.92, $P=4.76$E–6) and southwest China (38.9 vs. 73.5%, OR = 4.67, 95% CI = 2.56–8.51, $P=4.89$E–7). There was no statistical difference in the mutation rates between MN from patients living in northwest China and southwest China ($\chi^2=1.14$, $P=0.284$). With regard to histological types of MN, there were statistically significant differences in mutation rates among intradermal (173 of 272, 63.6%), compound (21 of 41, 51.2%), and junctional (10 of 28, 35.7%) MN ($\chi^2=9.65$, $P=8.02$E–3).

As shown in Table 3, the mutation rate in congenital MN ($N=104$) was similar to that of acquired MN ($N=152$; 58.7 vs. 59.2%) in the overall cohort and in the three geographic regions (χ^2 test, all $P>0.05$). A statistically significant difference in mutation rates was seen in congenital MN among the three regions ($\chi^2=10.38$, $P=5.58$E–3). By multiple logistic regression analysis, the mutation rate was significantly lower in those with congenital MN from northeast China than in those from northwest (OR = 2.81, 95% CI = 1.08–7.29, $P=0.034$) and southwest China (OR = 4.83, 95% CI = 1.70–13.76, $P=3.18$E–3). Similarly, there was a statistically significant difference in mutation rate in acquired MN among the three regions ($\chi^2=19.21$, $P=6.73$E–5). The rate was significantly lower in acquired MN from northeast China than in that from northwest (OR = 4.33, 95% CI = 1.76–10.68, $P=1.44$E–3) and southwest China (OR = 6.71, 95% CI = 2.72–16.58, $P=3.66$E–5).

Two groups of patients with MN sought biopsy or removal of their lesions. One group of patients ($N=108$) had early warning signs of MM, including itching, pain, broken skin, an increase in nevus size, changes in coloration, and appearance of new lesions (Friedman *et al.*, 1985). The other group ($N=159$), in whom there were no early warning signs, sought to remove the MN for cosmetic reasons or out of fear of potential melanoma development. The mutation rate in patients with and without early signs was 56.5% (61 of 108 cases) and 59.7% (95 of 159 cases), respectively, which was not statistically different ($\chi^2=0.28$, $P=0.59$). Although no unambiguous MM was defined in this cohort of samples, seven patients with warning signs had intradermal nevus with mild dysplastic hyperplasia. Four of these cases harbored the *BRAF* mutation.

A statistically significant difference in mutation rates was found among the decadal ages of onset in patients with acquired MN ($\chi^2=13.23$, $P=0.021$). Interestingly, the mutation rate in patients with acquired MN peaked during the third decade of life (27 of 38, 71.1%), an observation that is in accordance with the incidence peak of MN in the general population.

Effect of UV radiation on *BRAF* mutation in MN of Chinese Han

People with MN living in regions with high UV intensity had much higher mutation rates than those living in low-intensity regions. To determine whether UV exposure has a cumulative effect on the *BRAF* mutation, we investigated the

Table 1. *BRAF* exon 15 T1799A mutation in the MN of Chinese Han

	Northeast China E: 123°24′ N: 41°45′ A: 45 m UVR: 0.079 J m^{-2}		Northwest China E: 101°45′ N: 36°38′ A: 2,295 m UVR: 0.164 J m^{-2}		Southwest China E: 102°41′ N: 25°01′ A: 1,896 m UVR: 0.174 J m^{-2}		Total no.	
Selected variable[1]	**Total no.**	**No. with mutation (%)**	**Total no.**	**No. with mutation (%)**	**Total no.**	**No. with mutation (%)**	**Total no.**	**No. with mutation (%)**
Total no.	113	44 (38.9)	115	77 (67.0)	113	83 (73.5)	341	204 (59.8)
Gender								
Male	43	15 (34.9)	55	33 (60.0)	36	26 (72.2)	134	74 (55.2)
Female	70	29 (41.4)	60	44 (73.3)	77	57 (74.0)	207	130 (62.8)
Duration of disease (years)								
⩽10	41	11 (26.8)	36	23 (63.9)	45	34 (75.6)	122	68 (55.7)
11–20	19	6 (31.6)	11	7 (63.6)	19	14 (73.7)	49	27 (55.1)
21–30	14	5 (35.7)	13	9 (69.2)	9	6 (66.7)	36	20 (55.6)
31–40	8	7 (87.5)	10	8 (80.0)	7	7 (100)	25	22 (88.0)
⩾41	6	3 (50.0)	7	4 (57.1)	4	3 (75.0)	17	10 (58.8)
Age of onset (years)								
At birth	42	17 (40.5)	32	21 (65.6)	30	23 (76.7)	104	61 (58.7)
0.1–10	7	2 (28.6)	5	3 (60.0)	7	4 (57.1)	19	9 (47.4)
11–20	8	4 (50.0)	9	5 (55.6)	14	11 (78.6)	31	20 (64.5)
21–30	7	1 (14.3)	14	12 (85.7)	17	14 (82.4)	38	27 (71.1)
31–40	12	5 (41.7)	8	7 (87.5)	10	9 (90.0)	30	21 (70.0)
⩾41	12	3 (25)	9	3 (33.3)	6	3 (50.0)	27	9 (33.3)
Anatomic site								
Constantly exposed	55	20 (36.4)	62	39 (62.9)	69	50 (72.5)	186	109 (58.6)
Intermittently exposed	39	19 (48.7)	36	30 (83.3)	32	28 (87.5)	107	77 (72.0)
Nonexposed	19	5 (26.3)	17	8 (47.1)	12	5 (41.7)	48	18 (37.5)
Danger signs								
Yes	33	12 (36.4)	36	23 (63.9)	39	26 (66.7)	108	61 (56.5)
No	70	28 (40.0)	46	30 (65.2)	43	37 (86.0)	159	95 (59.7)
Histogenetic type								
IN	89	38 (42.7)	86	63 (73.3)	97	72 (74.2)	272	173 (63.6)
JN	13	3 (23.1)	10	5 (50.0)	5	2 (40.0)	28	10 (35.7)
CN	11	3 (27.3)	19	9 (47.4)	11	9 (81.8)	41	21 (51.2)

Abbreviations: A, altitude; *BRAF*, *v-raf murine sarcoma viral oncogene homolog B*; CN, compound nevus; E, east longitude; IN, intradermal nevus; JN, junctional nevus; MN, melanocytic nevus; N, north latitude.
[1]The sum of subjects in each subgroup may be less than the total number of subjects because some subjects did not provide the information.

Table 2. Description of mutations detected other than *BRAF* exon 15 T1799A

Region	Case no.	Gender	Age (year)	Congenital vs. acquired	Warning signs	Histogenetic type	Site of occurrence	*BRAF* exon 15	*NRAS* exon 3
BRAF mutation									
Northeast China	1.1.1	Male	65	Acquired	Yes	AL	Foot	A1781G (D594G)	wt
Southwest China	1.3.1	Female	27	Congenital	Yes	IN	Lower jaw	T1799A G1800A (V600E)	wt
NRAS mutation									
Northeast China	2.1.1	Female	28	Congenital	No	IN	Lower jaw	wt	A182G (Q61R)
Northwest China	2.2.1	Male	14	Not recorded	Not recorded	LMM	Neck	wt	A182G (Q61R)
	2.2.2	Male	18	Congenital	No	IN	Scalp	T1799A (V600E)	G187T (E63 stop codon)
Southwest China	2.3.1	Male	14	Acquired	Yes	IN	Labrum	T1799A (V600E)	C235A (L79I)

Abbreviations: AL, acral lentiginous; *BRAF*, *v-raf murine sarcoma viral oncogene homolog B*; CN, compound nevus; F, female; IN, intradermal nevus; JN, junctional nevus; LMM, lentigo maligna melanoma; M, male; wt, wild type.

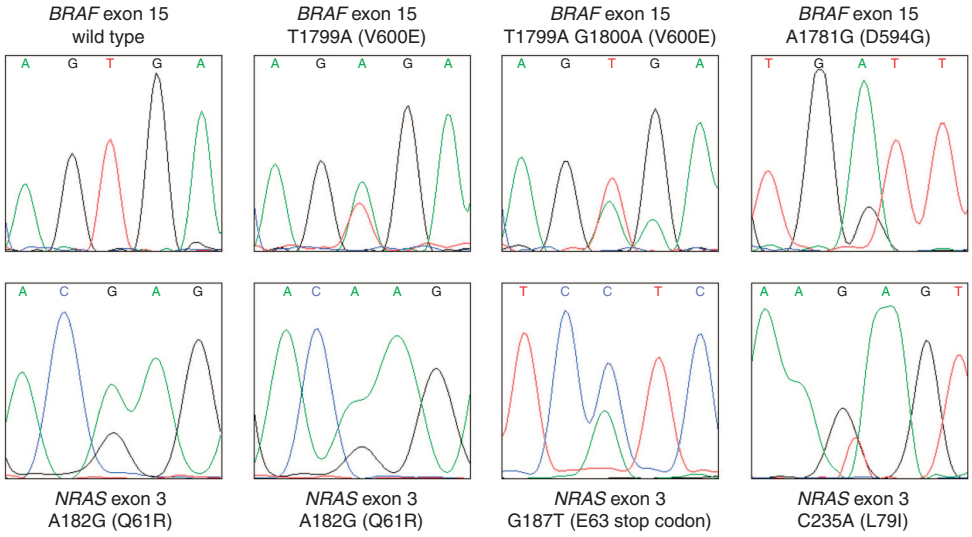

Figure 1. Representative mutations detected in this study.

presence and severity of solar elastosis in the perilesional skin of MN. We detected recognizable solar elastosis in 23 of 282 MN (8.15%). There was no statistical difference in the mutation rates between those with solar elastosis and those without ($\chi^2 = 0.04$, $P = 0.84$).

Because different anatomical sites receive different levels of solar exposure, they can be classified into constantly sun-exposed sites, intermittently exposed sites, and nonexposed sites (Maldonado *et al.*, 2003). In the overall samples of MN, there were statistically significant differences in mutation rates among patients who received different patterns of UV exposure ($\chi^2 = 16.63$, $P = 2.45E{-}4$). MN on intermittently exposed sites had higher mutation rates than those on constantly sun-exposed sites ($\chi^2 = 5.23$, $P = 0.022$) and nonexposed sites ($\chi^2 = 16.59$, $P = 4.65E{-}5$). As determined using multiple logistic regression analysis, MN on intermittently exposed sites had a higher rate of *BRAF* mutation than those on nonexposed sites (OR = 3.31, 95% CI = 1.41–7.77, $P = 5.83E{-}3$), whereas there was no statistical difference in mutation rates between patients with MN on nonexposed sites and constantly exposed sites (OR = 1.67, 95% CI = 0.76–3.66, $P = 0.20$).

Acquired MN ($N = 152$) on intermittently exposed sites had a statistically significant higher mutation rate than those

Table 3. *BRAF* exon 15 T1799A mutation in acquired and congenital melanocytic nevi

	Northeast China				Northwest China				Southwest China				Total no.			
	Congenital		Acquired		Congenital		Acquired		Congenital		Acquired		Congenital		Acquired	
Selected variable[1]	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)
Total no.	42	17 (40.5)	47	16 (34.0)	32	21 (65.6)	51	33 (64.7)	30	23 (76.7)	54	41 (75.9)	104	61 (58.7)	152	90 (59.2)
Gender																
Male	14	3 (21.4)	16	7 (43.8)	16	10 (62.5)	27	17 (63.0)	9	8 (88.9)	16	13 (81.3)	39	21 (53.8)	59	37 (62.7)
Female	28	14 (50.0)	31	9 (29.0)	16	11 (68.8)	24	16 (66.7)	21	15 (71.4)	38	28 (73.7)	65	40 (61.5)	93	53 (57.0)
Duration of lesions (years)																
⩽10	6	0	35	11 (31.4)			36	23 (63.9)	3	2 (66.7)	42	32 (76.2)	9	2 (22.2)	113	66 (58.4)
11–20	9	2 (22.2)	10	4 (40.0)	5	2 (40.0)	6	5 (83.3)	9	6 (66.7)	10	8 (80.0)	23	10 (43.5)	26	17 (65.4)
21–30	13	5 (38.5)	1	0	10	7 (70.0)	3	2 (66.7)	8	6 (75.0)	1	0	31	18 (58.1)	5	2 (40.0)
31–40	8	7 (87.5)			10	8 (80.0)			6	6 (100)	1	1 (100)	24	21 (87.5)	1	1 (100)
⩾41	6	3 (50.0)			7	4 (57.1)			4	3 (75.0)			17	10 (58.8)		
Age of onset (years)																
At birth	42	17 (40.5)			32	21 (65.6)			30	23 (76.7)			104	61 (58.7)		
⩽10			7	2 (28.6)			5	3 (60.0)			7	4 (57.1)			19	9 (47.4)
11–20			8	4 (50.0)			9	5 (55.6)			14	11 (78.6)			31	20 (64.5)
21–30			7	1 (14.3)			14	12 (85.7)			17	14 (82.4)			38	27 (71.1)
31–40			12	5 (41.7)			7	6 (85.7)			10	9 (90.0)			30	21 (70.0)
⩾41			12	3 (25.0)			9	3 (33.3)			6	3 (50.0)			27	9 (33.3)
Anatomic location/site																
Constantly exposed	22	8 (36.4)	22	7 (31.8)	17	9 (52.9)	24	15 (62.5)	20	17 (85.0)	28	20 (71.4)	59	34 (57.6)	74	42 (56.8)
Intermittently exposed	14	6 (42.9)	15	7 (46.7)	12	10 (83.3)	17	14 (82.4)	7	6 (85.7)	19	16 (84.2)	33	22 (66.7)	51	37 (72.5)
Nonexposed	6	3 (50.0)	10	2 (20.0)	3	2 (66.7)	10	4 (40.0)	3	0	7	5 (71.4)	12	5 (41.7)	27	11 (40.7)
Warning signs																
Yes	12	5 (41.7)	17	6 (35.3)	11	7 (63.6)	19	10 (52.6)	10	7 (70.0)	20	16 (80.0)	33	19 (57.6)	56	32 (57.1)
No	27	12 (44.4)	29	10 (34.5)	16	10 (62.5)	22	15 (68.2)	11	9 (81.8)	24	20 (83.3)	54	31 (57.4)	75	45 (60.0)
Solar elastosis																
No	39	14 (35.9)	42	15 (35.7)	19	13 (68.4)	29	20 (69.0)	22	17 (77.3)	38	29 (76.3)	80	44 (55.0)	109	64 (58.7)
Yes	1	1 (100)	3	1 (33.3)	1	0	4	1 (25.0)	2	2 (100)	9	7 (77.8)	4	3 (75.0)	16	9 (56.3)
Histogenetic type																
IN	34	16 (47.1)	39	13 (33.3)	23	16 (69.6)	37	27 (73.0)	24	18 (75.0)	46	35 (76.1)	81	50 (61.7)	122	75 (61.5)
JN	4	1 (25.0)	5	1 (20.0)	5	3 (60.0)	3	1 (33.3)	1	0	4	2 (50.0)	10	4 (40.0)	12	4 (33.3)
CN	4	0	3	2 (66.7)	4	2 (50.0)	11	5 (45.5)	5	5 (100)	4	4 (100)	13	7 (53.8)	18	11 (61.1)

Abbreviations: *BRAF, v-raf murine sarcoma viral oncogene homolog B*; CN, compound nevus; IN, intradermal nevus; JN, junctional nevus.
[1]The sum of subjects in each subgroup may be less than the total number of subjects because some subjects did not provide the information.

Table 4. Patients with multiple MN lesions

Region	Case no.	Gender	Age (year)	Congenital or acquired	Warning signs	Histogenetic types	Site of occurrence	*BRAF* exon 15	*NRAS* exon 3
Northeast China	1.1	Female	22	Congenital	No	IN	Scalp–nasolabial fold	wt–M	wt
	1.2	Female	30	Uncertain	No	IN	Abdomen–nasolabial fold–lip	M–M–M	wt
	1.3	Female	66	Acquired	Yes	IN	Back–prototHorax	wt–M	wt
	1.4	Female	19	Acquired	No	IN	Waist–back	M–M	wt
	1.5	Female	28	Acquired	No	IN	Face–lower jaw–nostril	wt–M–M	wt
	1.6	Female	23	Congenital	No	IN	Face–arm	wt–wt	wt
	1.7	Female	31	Acquired	Yes	JN	Abdomen–buttocks	wt–wt	wt
	1.8	Female	16	Uncertain	Uncertain	CN–IN	Foot–thigh	M–M	wt
	1.9	Female	27	Acquired	No	CN–IN	Interdigit–dorsum manus	wt–wt	wt
Northwest China	2.1	Male	47	Acquired	No	IN	Occiput–occiput	wt–wt	wt
	2.2	Male	70	Acquired	No–yes	IN–CN	Shoulder–neck	M–wt	wt
	2.3	Female	46	Acquired	Yes–no	IN	Nasal ala–preauricula	wt–wt	wt
Southwest China	3.1	Female	23	Uncertain	Uncertain	IN	Umbilicus–arm	M–M	wt
	3.2	Female	32	Acquired	Yes	JN	Finger pulp–planta pedis	M–M	wt
	3.3	Female	32	Acquired	No	IN	Post aurem–abdomen 1,2	M–M–M	wt
	3.4	Female	46	Acquired	Uncertain	IN	Forehead–waist	M–wt	wt
	3.5	Female	30	Acquired	Yes	IN	Neck–waist–abdomen	M–M–M	wt
	3.6	Female	29	Congenital	No–Yes	IN	Foot–leg	wt–wt	wt
	3.7	Female	40	Acquired	No	IN	Face–neck	M–M	wt
	3.8	Male	34	Acquired	Yes	IN–CN	Prototthorax–axilla	wt–wt	wt

Abbreviations: *BRAF, v-raf murine sarcoma viral oncogene homolog B*; CN, compound nevus; IN, intradermal nevus; JN, junctional nevus; M, mutant type; MN, melanocytic nevus; *NRAS, neuroblastoma rat sarcoma oncogene*; wt, wild type.

on constantly exposed and nonexposed sites ($\chi^2 = 7.76$, $P = 0.02$). MN on intermittently exposed sites harbored higher mutation rates than those on nonexposed sites (OR = 3.58, 95% = 1.22–10.50, $P = 0.019$); MN on constantly exposed sites also harbored higher rates of mutation than those on nonexposed sites, but the difference was not statistically significant (OR = 1.61, 95% CI = 0.60–4.32, $P = 0.34$). Intriguingly, when the cohort of congenital MN ($n = 104$) was analyzed, there was no statistical difference in mutation rates among MN from patients who might have received different patterns of UV exposure ($\chi^2 = 2.33$, $P = 0.31$; Table 3).

Status of *BRAF* exon 15 T1799A mutation in patients with multiple biopsied samples

Of the 20 patients who offered two or three MN samples, as shown in Table 4, 75% (15 of 20 patients) had the same *BRAF* status in all biopsies. Five patients (25%) had both wild and mutant types of the *BRAF* mutation. It seemed that patients with multiple nevi were inclined to have either the wild type or the mutant type of *BRAF* mutation, underlining a constitutional predilection in the occurrence of *BRAF* mutation.

Chinese Han MMs harbored a low frequency of *BRAF* T1799A mutation

Of the 195 cases of MM, 180 yielded DNA product for analysis, as shown in Table 5. Of 180 MMs, 27 (15.0%) contained the *BRAF* mutation. No statistically significant difference in mutation rate was seen between MMs from northeast China (13.3%) and those from northwest China (16.9%; $\chi^2 = 0.48$, $P = 0.79$). The mutation rates were not affected by gender, age of onset, pattern of UV exposure, histological type of MM, or depth of invasive growth as measured by Breslow thickness (χ^2 test, all $P > 0.05$).

Thirty-six patients with MM reported a preexisting MN. Five of these (13.9%) had a *BRAF* mutation. There was no significant difference in mutation rate between MMs with preexisting MN and those without MN ($\chi^2 = 0.03$, $P = 0.86$). Six cases reported a previous history of trauma before the onset of MM, and none of these harbored the mutation.

DISCUSSION

Ever since the *BRAF* T1799A mutation was implicated in MM (Davies *et al.*, 2002), reports of variation in mutation rates (20–80%) have accumulated in the literature (Davies *et al.*, 2002; Lang and MacKie, 2005; Akslen *et al.*, 2008).

Table 5. *BRAF* exon 15 T1799A mutation in the MM of Chinese Han

	Northeast China		Northwest China		Southwest China		Total no.	
Selected variable[1]	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)	Total no.	No. with mutation (%)
Total no.	83	11 (13.3)	89	15 (16.9)	8	1 (12.5)	180	27 (15.0)
Gender								
Male	45	8 (17.8)	54	9 (16.7)	4	0	103	17 (16.5)
Female	38	3 (7.9)	35	6 (17.1)	4	1 (25.0)	77	10 (13.0)
Age of onset (years)								
⩽30	1	0	3	0			4	0
31–40	5	1 (20.0)	4	1 (25.0)			9	2 (22.2)
41–50	13	2 (15.4)	8	2 (25.0)	1	0	22	4 (18.2)
51–60	13	3 (23.1)	5	0			18	3 (16.7)
61–70	15	1 (6.7)	20	5 (25.0)	3	0	38	6 (15.8)
⩾70	11	1 (9.1)	10	0	1	0	22	1 (4.5)
Anatomic site								
Constantly exposed	16	1 (6.3)	22	5 (22.7)	4	1 (25.0)	42	7 (16.7)
Intermittently exposed	8	1 (12.5)	10	2 (20.0)	0	0	18	3 (16.7)
Nonexposed	59	9 (15.3)	57	8 (14.0)	4	0	120	17 (14.2)
Preexisting nevus								
Yes	18	2 (11.1)	14	2 (14.3)	4	1 (25.0)	36	5 (13.9)
No	42	6 (14.3)	42	7 (16.7)	2	0	86	13 (15.1)
Solar elastosis								
No	52	6 (11.5)	48	9 (18.8)	4	0	104	15 (14.4)
Yes	4	0	2	1 (50)	1	0	7	1 (14.3)
Histogenetic type								
AL	62	9 (14.5)	60	11 (18.3)	5	0	127	20 (15.7)
SSM	9	2 (22.2)	6	2 (33.3)	2	0	17	4 (23.5)
LMM	3	0	3	1 (33.3)	0	0	6	1 (16.7)
NM	7	0	5	1 (20.0)	1	1 (100)	13	2 (15.4)
Melanoma								
Melanoma *in situ*	15	3 (20.0)	8	1 (12.5)	0	0	23	4 (17.4)
Invasive melanoma	68	8 (11.8)	81	14 (17.3)	8	1 (12.5)	157	23 (14.6)

Abbreviations: AL, acral lentiginous melanoma; *BRAF*, *v-raf murine sarcoma viral oncogene homolog B*; LMM, lentigo maligna melanoma; MM, malignant melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma.
[1]The sum of subjects in each subgroup may be less than the total number of subjects because some subjects did not provide the information.

Detection of the *BRAF* T1799A mutation in the MN, albeit in varying mutation rates, challenged the role of the *BRAF* mutation in the ontogenesis of MM. The varying detection rate of the *BRAF* T1799A mutation in MM and MN has been attributed to a variety of parameters, e.g., sample size, histological type, pattern of UV exposure, stage of progression, anatomic site of the lesion, and the particular techniques used (Shinozaki *et al.*, 2004; Liu *et al.*, 2007; Wu *et al.*, 2007; Besaratinia and Pfeifer, 2008). However, most of the studies were conducted on samples from

light-skinned populations; very few data are available from Asian populations (Takata and Saida, 2006). The present study enrolled what is so far the largest cohort of Chinese Han patients with MM and MN from regions representing different geographical conditions, thus providing a more comprehensive analysis for factors that might correlate with the *BRAF* T1799A mutation. All the patients were of Chinese Han ethnicity and had skin types III and IV, ensuring a relatively homogeneous genetic background. A major drawback of the study was that we were unable to collect a sufficient number of MM samples from southwest China because of administrative restrictions.

Functional mutations in the RAS/mitogen-activated protein kinase pathway may lead to cell proliferation (Fecher *et al.*, 2008). Mutations in *NRAS* exons 2 and 3, *BRAF* exon 11, and, most prevalently, *BRAF* exon 15 T1799A have been reported in MN and MM (Poynter *et al.*, 2006; Saldanha *et al.*, 2006). In our first-phase study, we did not find mutations in *BRAF* exon 11 and *NRAS* exon 2 in a total of 280 cases of MN and MM from Chinese Han. We detected only 4 cases (out of 274 samples) with a mutation in *NRAS* exon 3. This is strikingly different from the reported data on MN and MMs from fair-skinned people (Papp *et al.*, 2005; Bauer *et al.*, 2007; Akslen *et al.*, 2008).

The overall *BRAF* T1799A mutation rate in Chinese MN was 59.8%, comparable to that in populations of other ethnicities (Pollock *et al.*, 2003; Poynter *et al.*, 2006). However, we noticed a very significant difference in mutation rates among the different Chinese regions. The three geographic regions that we studied have different altitudes, latitudes, and intensities of annual solar radiance (Gong *et al.*, 1992). In our study, we found that acquired MN from regions with higher UV intensity harbored higher mutation rates in *BRAF* exon 15 T1799A. An interesting finding was that the same trend was observed for congenital MN, the occurrence of which should not be affected by solar exposure. We postulate that either there are different pathogenesis pathways in congenital and acquired MN or that UV exposure has an indirect role in promoting the *BRAF* mutation.

Several studies have reported frequent *BRAF* mutations in congenital MN (Papp *et al.*, 2005; Wu *et al.*, 2007). Others have reported that congenital MN harbor a low rate of *BRAF* mutations but a high rate of *NRAS* mutations (Bauer *et al.*, 2007; Dessars *et al.*, 2009). We found that 58.7% of congenital MN harbored a *BRAF* mutation, whereas few samples harbored the *NRAS* mutation. Furthermore, the rate of *BRAF* mutation in congenital MN was influenced only by regional variation and not by the other documented variables such as age, gender, duration of the disease, or pattern of solar exposure. The underlying causes of the *BRAF* mutation in congenital MN from Chinese Han remain elusive.

Solar exposure is the most common factor in acquired MN development. Studies have shown that ambient UV exposure at early stages of life contributes to the *BRAF* mutation in MN, especially when patients are intermittently exposed to solar radiation (Thomas *et al.*, 2007). Scoring of solar elastosis was considered a reliable histological method in recording the accumulated effect of UV exposure (Maldonado *et al.*, 2003; Landi *et al.*, 2006). For Chinese Han patients with skin type III or IV, only a minor portion of samples showed recognizable signs of solar elastosis. Patients with acquired MN on sites that might receive intermittent ambient UV exposure harbored a higher rate of *BRAF* mutations than those on nonexposed and constantly exposed sites. The *BRAF* mutation might account for the occurrence of a proportion of acquired MN, although there may be other elusive determinants.

In this study, MN with previous warning signs harbored mutation rates similar to those for whom there were no such signs, suggesting that the perturbed clinical course in MN did not contribute to *BRAF* mutation. In the MM cohort, there were 36 patients with a previous history of *de novo* MN. The mutation rate was 13.9%, comparable to that in patients with primary MM. Taken together, we speculate that the *BRAF* T1799A mutation is an initial event in melanocytic hyperplasia but not a decisive event in melanocyte carcinogenesis (Pollock *et al.*, 2003).

Multiple nevi from the same patient tended to have a concurrent state of *BRAF* mutation (Table 4). Similar findings were reported by Kumar *et al.* (2004). The findings indicated that a genetic predisposition for the somatic *BRAF* mutation exists in MN. Germline mutation was not detected in ~4,000 individuals (MM patients and controls) in a single study (James *et al.*, 2006) nor in 80 independent melanoma-prone families or patients with multiple primary melanoma without a familial history (Laud *et al.*, 2003).

MM is relatively rare in Chinese Han. Among the four major histological types of MM, acral lentiginous melanoma is the most prevalent (Sun *et al.*, 2003). The *BRAF* mutation was detected in 15% of Chinese Han patients with MM, a moderate mutation rate similar to that observed in Japanese studies. Most of the MM samples were collected from northeast China and northwest China (171 cases). Although the geographical conditions were quite distinct between these two regions, we found no difference in mutation rate between the two groups. Furthermore, the mutation rate in samples from these two regions was not affected by any of the categorical variables listed in Table 3. Our results suggest that *BRAF* mutations (as well as *NRAS* mutations) have a smaller role in the carcinogenesis of MM in Chinese Han than in Western patients and that other genetic abnormalities might be involved in the development of MM (Landi *et al.*, 2006; Dankort *et al.*, 2009; Yu *et al.*, 2009). It seems that there is a higher penetrance of *BRAF* mutations in the progression from MN to MM in Caucasians versus Chinese Han. The possible existence of polymorphisms in modifier genes may account for these findings, as suggested in two recent studies (Demenais *et al.*, 2010; Dworkin *et al.*, 2010). However, this issue has not yet been addressed in the MMs of Chinese Han.

MATERIALS AND METHODS

Sources of samples

Formalin-fixed paraffin-embedded tissue blocks histologically diagnosed as MN and MM were retrieved from several reference hospitals located in northeast China, northwest China, and southwest China. The geographical conditions are described in Table 1 (Gong *et al.*, 1992). In total, we collected 379 cases of MN and 195 cases of

MM. In addition, we collected 20 cases in which more than two biopsied MN samples from different anatomic locations were collected (Table 4). Congenital nevi were defined as nevi that are present at birth (Bauer *et al.*, 2007). All samples were from local inhabitants of Chinese Han. Available demographic information was recorded from medical records. Patient consent for experiments was not required because patients consented to the storage and use of their tissue left over from surgery at the disposal of the hospitals; research use of such samples is lawful in China. The study was approved by the institutional review board of China Medical University and was in accordance with the Declaration of Helsinki Principles.

Histological evaluation of UV exposure

As described, representative areas of solar elastosis on hematoxylin and eosin–stained sections of normal skin surrounding the MN and MM were examined (Maldonado *et al.*, 2003; Landi *et al.*, 2006). Dichotomous categorization of the presence or absence of solar elastosis was used for grading.

Laser capture microdissection and DNA extraction

Five to 10 consecutive 8 μm-thick sections were cut and stained with hematoxylin. Using a laser capture microdissection system (MMI, Glattbrugg, Switzerland), nevus and melanoma cells were microdissected and collected. DNA was extracted using the QIAGEN QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

PCR and allele-specific PCR

PCR and allele-specific PCR were carried out with the GeneAmp PCR System 9700 (PerkinElmer, Oak Brook, IL). The PCR conditions employed were as previously described (Davies *et al.*, 2002; Lang and MacKie, 2005).

Sequencing

All sequencing was performed on an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA). Mutations were detected using Chromas 2.31, Technelysium Pty, Queensland, Australia. All chromatograms were also manually reviewed to confirm the mutations.

Statistical analysis

All data were entered into SPSS (version 15.0, SPSS, Chicago, IL) for statistical analysis. Categorical data were analyzed using the χ^2 test or Fisher's exact test. Multivariable logistic regression analyses were performed to obtain the OR and 95% CI. For each selected variable of interest, variables such as region, gender, age of diagnosis, histological type, or UV exposure pattern were included for adjustment whenever appropriate. Owing to the small number of MM cases (eight) from southwest China, the statistical analyses on MM were performed mainly on cohorts from northeast and northwest China. A two-tailed $P<0.05$ was considered statistically significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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